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Chlorinated Compounds Found in Waste-Treatment Effluents and Their Capacity to Bioaccumulate

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16. ABSTRACT

The incorporation of chlorine into compounds during the disinfection of waste effluents with chlorine is an undesirable end result of effluent treatment due to an increase in the persistance and bioaccumulatability of the chloro-organics. The problems that arise with bioaccumulation of organic compounds are generally not immediately apparent, and a major segment of the ecosystem may be contaminated before the problem is recognized. A discussion of chlorinated compounds found in waste-treatment effluents and their capacity to bioaccumulate is presented. Attention is especially drawn to an unreported compound type, the chloroanisoles.

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ABSTRACT

As part of an ongoing research program to assess possible long-term environmental effects due to the formation of stable reaction products during disinfection processes, fish (fathead minnows, *Pimephales promelas*) and water from the 9-month chronic toxicity tests at two wastewater treatment plants in Michigan are being analyzed for chemical residues at this laboratory (formerly named the National Water Quality Laboratory).

Gel permeation chromatography was used for sample clean-up and gas chromatography/mass spectrometry was used for sample analysis. Di- and trichlorophenols, di- and trichlorobenzenes, and trichloroanisoles were either not detected or detected at lower levels in the fish from nondisinfected effluent exposures compared to fish exposed to chlorinated effluent. Tetra- and pentachlorophenols, PCB's, DDT's, toxaphene components, chlordane, and nonachlor were found in all fish raised in the sewage effluent. Tribromoanisole was tentatively identified in fish that lived in BrCl-treated wastewater.

Reports appear to be conclusive in support of the argument that even the most gentle chlorination conditions will cause chlorine to be incorporated into organic molecules. The incorporation of chlorine into an organic molecule increases its lipophilic character and at the same time normally causes an increase in the observed toxicity or bioaccumulation, or both.

The persistence of these compounds is now becoming a concern. Not all organochlorine compounds bioaccumulate to high levels. The data

suggests that polar compounds are more easily biodegraded, and the nonpolar (highly lipophilic) compounds accumulate. Some investigators have been able to demonstrate positive correlation between the *n*-octanol/water partition coefficients for given compounds and their ability to bioaccumulate in various species of fish.

Chlorination has been used extensively for disinfection of waste effluents by both industry and municipalities. In addition, it was assumed that chlorine was oxidizing the organic compounds to innocuous substances (CO_2, H_2O, and easily degradable organics) and thereby lower the BOD of the effluent. It has now been shown that although chlorine does oxidize many compounds, it also creates many organochlorine compounds which are more toxic. Carlson (1975) has shown that chlorinated phenols lower the BOD by destroying the bacteria. Carlson et al. (1975) have also shown that phenol incorporates chlorine very readily over a wide range of pH, while under conditions similar to disinfection (Table 1).

Table 1. Percentage of available chlorine incorporated by various compounds treated with 7×10^{-4} *M* aqueous chlorine at three pH levels (3, 7, and 10) for 20 minutes, at 25°C[a]

	pH		
Compound[b]	3	7	10
Phenol	97.8	97.6	97.6
Anisole	80.7	11.4	2.8
Acetanilide	55.3	3.4	-
Toluene	11.1	2.9	-
Benzyl alcohol	2.3	-	-
Benzonitrile	2.1	-	-
Nitrobenzene	1.8	-	-
Chlorobenzene	1.8	-	-
Methylbenzoate	1.8	-	-
Benzene	1.5	-	-

[a] R. M. Carlson et al. (1975).

[b] Aqueous concentration = $9.5 \pm 0.6 \times 10^{-4}$ *M*.

Chlorination of water supplies has received considerable attention (Jolley 1973, 1975, Jolley et al. 1975, Glaze and Henderson 1975, Glaze et al. 1973, Bellar et al. 1974) in the last few years (Tables 2, 3, and 4). People are becoming concerned not only with the production of toxic materials but also with the production of possible carcinogens.

Table 2. Tentative identification and concentration of chlorine-containing constituents in chlorinated effluents (Oak Ridge, Tennessee) by liquid chromatographic techniques[a]

Organic compound	Concentration (μg/liter)
5-chlorouracil	4.3
5-chlorouridine	1.7
8-chlorocaffeine	1.7
6-chloroguanine	0.9
8-chloroxanthine	1.5
2-chlorobenzoic acid	0.26
5-chlorosalicylic acid	0.24
4-chloromandelic acid	1.1
2-chlorophenol	1.7
4-chlorophenylacetic acid	0.38
4-chlorobenzoic acid	1.1
4-chlorophenol	0.69
3-chlorobenzoic acid	0.62
3-chlorophenol	0.51
4-chlororesorcinol	1.2
3-chloro-4-hydroxybenzoic acid	1.3
4-chloro-3-methylphenol	1.5

[a]Jolley (1975).

To answer some of the questions being raised a program was initiated in Grandville and Wyoming, Michigan, to observe the effect of various disinfection processes upon the chemistry of organic molecules in municipal waste effluent. The four treatments examined were (1) chlorination, (2) chlorination followed by dechlorination with SO_2 treatment, (3) ozonation, and (4) chlorobromination (Fig. 1).

Table 3. Chlorinated organics in wastewater effluent (Denton, Texas) identified by gas chromatography/mass spectrometry methods*

Compound[a]	Concentration[b] (µg/l)
Chloroform[c]	—
Dibromochloromethane[c]	--
Dichlorobutane (-)[d]	27
3-chloro-2-methylbut-1-ene[e]	285
Chlorocyclohexane (118)[d]	20
Chloroalkyl acetate (-)[d]	—
o-dichlorobenzene[c]	10
Tetrachloroacetone[e]	11
p-dichlorobenzene[c]	10
Chloroethylbenzene[e]	21
Pentachloroacetone[c]	30
Hexachloroacetone[c]	30
Trichlorobenzene[c]	—
Dichloroethylbenzene[c]	20
Chlorocumene (154)[d]	--
N-methyl-trichloroaniline (209)[d]	10
Dichlorotoluene[e]	--
Trichlorophenol[e]	—
Chloro-α-methyl benzyl alcohol[e]	--
Dichloromethoxytoluene[e]	32
Trichloromethylstyrene (220)[d]	10
Trichloroethyl benzene (208)[d]	12
Dichloro-α-methyl benzyl alcohol (190)[d]	10
Dichloro-bis(ethoxy)benzene (220)[d]	30
Dichloro-α-methyl benzyl alcohol (190)[d]	—
Trichloro-α-methyl benzyl alcohol[e]	25
Tetrachlorophenol[c]	30
Trichloro-α-methyl benzyl alcohol[e]	50
Trichlorocumene (222)[d]	--
Tetrachloroethylstyrene (268)[d]	--
Trichlorodimethoxybenzene (240)[d]	--
Tetrachloromethoxytoluene (258)[d]	40
Dichloroaniline derivative (205)[f]	13
Dichloroaromatic derivative (249)[f]	15
Dichloroacetate derivative (203)[f]	20
Trichlorophthalate derivative (296)[f]	--
Tetrachlorophthalate derivative (340)[f]	--

*Glaze and Henderson 1975.

[a]Compounds may be listed more than once if gas chromatographic retention times indicate distinct positional isomers.

[b]Quantitative values should only be considered as estimates because explicit recovery data are not available for the authors' extraction system.

[c]Completed identification based on mass spectrometric interpretation and confirmed by comparison with a reference spectrum.

[d]Fragmentation pattern tentatively suggests proposed compound; probable molecular weight indicated in parentheses.

[e]Probable identification based on mass spectral interpretation.

[f]Mass spectral information too incomplete to propose a structure; probable molecular weight indicated in parentheses.

Table 4. Concentration of organochlorine compounds (μg/liter) in water from sewage treatment plants (several cities) measured by gas chromatography/mass spectrometry techniques[a]

Compound	Influent before treatment	Effluent before chlorination	Effluent after chlorination
Methylene chloride	8.2	2.9	3.4
Chloroform	9.3	7.1	12.1
1,1,1-trichloroethane	16.5	9.0	8.5
1,1,2-trichloroethylene	40.4	8.6	9.8
1,1,2,2-tetrachloroethylene	6.2	3.9	4.2
Σ Dichlorobenzenes	10.6	5.6	6.3
Σ Trichlorobenzenes	66.9	56.7	56.9

[a] T. A. Bellar et al. (1974).

One of the main objectives of this study was to investigate the chlorinated effluent for newly formed chloro compounds. It has been shown that phenols will incorporate chlorine under the conditions normally encountered in water treatment plants (Carlson et al. 1975). The number of chlorines incorporated depends on the reactivity of the particular phenol toward electrophilic attack. Phenol itself was not identified in either the water or the fish; however, dichloro- through pentachlorophenols were identified. An attempt was made to identify as many organics as possible by using the GC/MS (gas chromatography/mass spectrometry) system. The usual chlorinated pesticides and PCB's were identified in the fish as well as many aromatic hydrocarbons (Table 5). Di- and trichlorobenzene were only observed in the fish reared in the chlorinated effluents (Fig. 2).

An unreported compound type, anisole, was identified in both the water and the fish. Analysis of the effluent indicated that the parent phenols were present in larger quantities than the corresponding anisoles, and the fish analysis showed that the anisoles had become the more abundant of the two. Figure 3 shows a representative sample of anisole data illustrated by one of these compounds, pentachloroanisole.

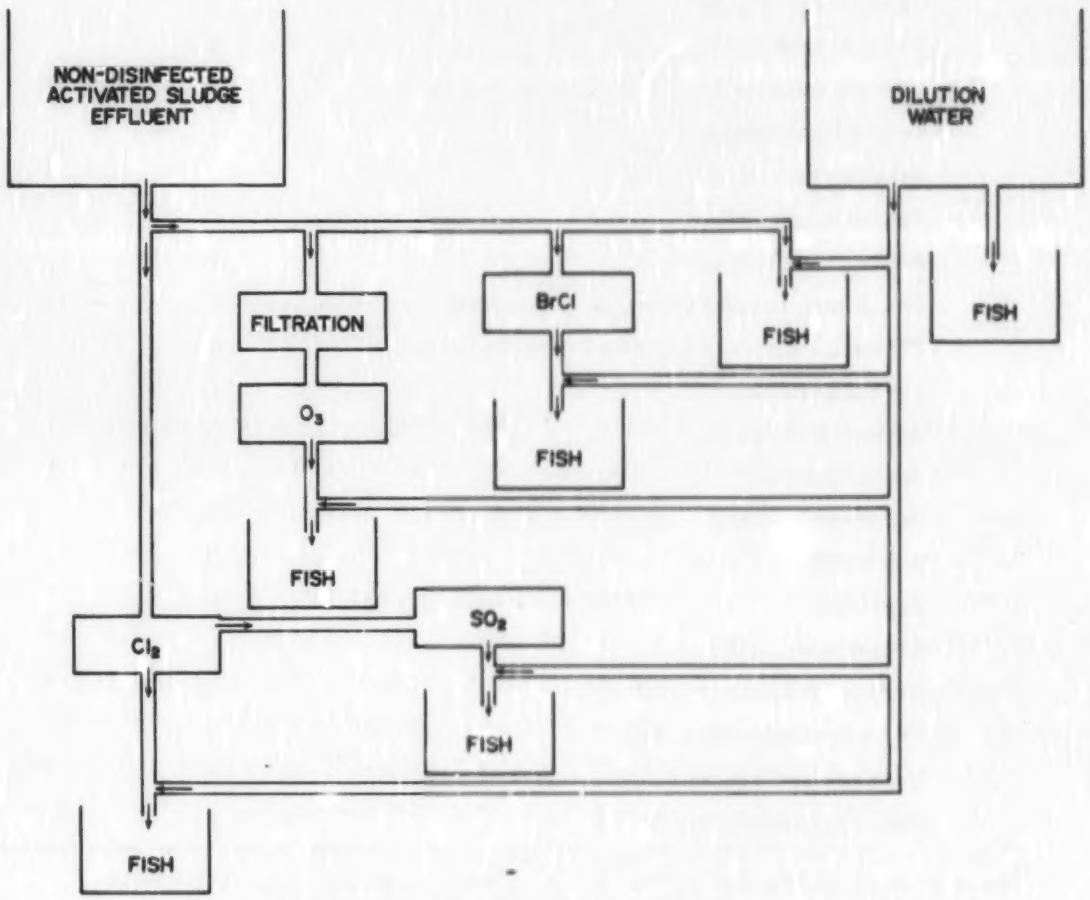

Fig. 1. Schematic diagram indicating the experimental disinfection processes used in the chronic bioassay tests at the Environmental Protection Agency's Grandville, Michigan, project.

Table 5. Chlorinated organic compounds identified by gas chromatography/mass spectrometry in fish raised in disinfected municipal effluent (Grandville, Michigan)

Compound	Identification[a]
Dichlorobenzene	A
Trichlorobenzene	A
Trichlorophenol	B
Trichloroanisole	B
Tetrachlorophenol	B
Tetrachloroanisole (2)	A/B
Pentachlorophenol	B
Pentachloroanisole	B
Trichloropropylmethoxynaphthalene	C
Tetrachloropropylmethoxynaphthalene	C
cis-chlordane	B
trans-chlordane	B
cis-nonachlor	B
trans-nonachlor	B
p,p'-DDE	B
p,p'-DDT	B
Tribromoanisole	A
Dichlorobiphenyl (3)	A
Trichlorobiphenyl (5)	A
Tetrachlorobiphenyl (6)	A
Pentachlorobiphenyl (5)	A

[a] A, identification based on MS; B, identification supported with retention time; C, fragmentation pattern suggests a compound containing groups indicated.

Experiments have not been carried out to determine whether or not activated sludge sewage treatment or fish possess the ability to methylate phenols; therefore, it is difficult to speculate on the bioconcentration factor of anisoles. Many of the PCB's and chlorinated pesticides were difficult to analyze using 36 liters of effluent; however, they were concentrated to such an extent that the analysis of 25 g of fish was not difficult.

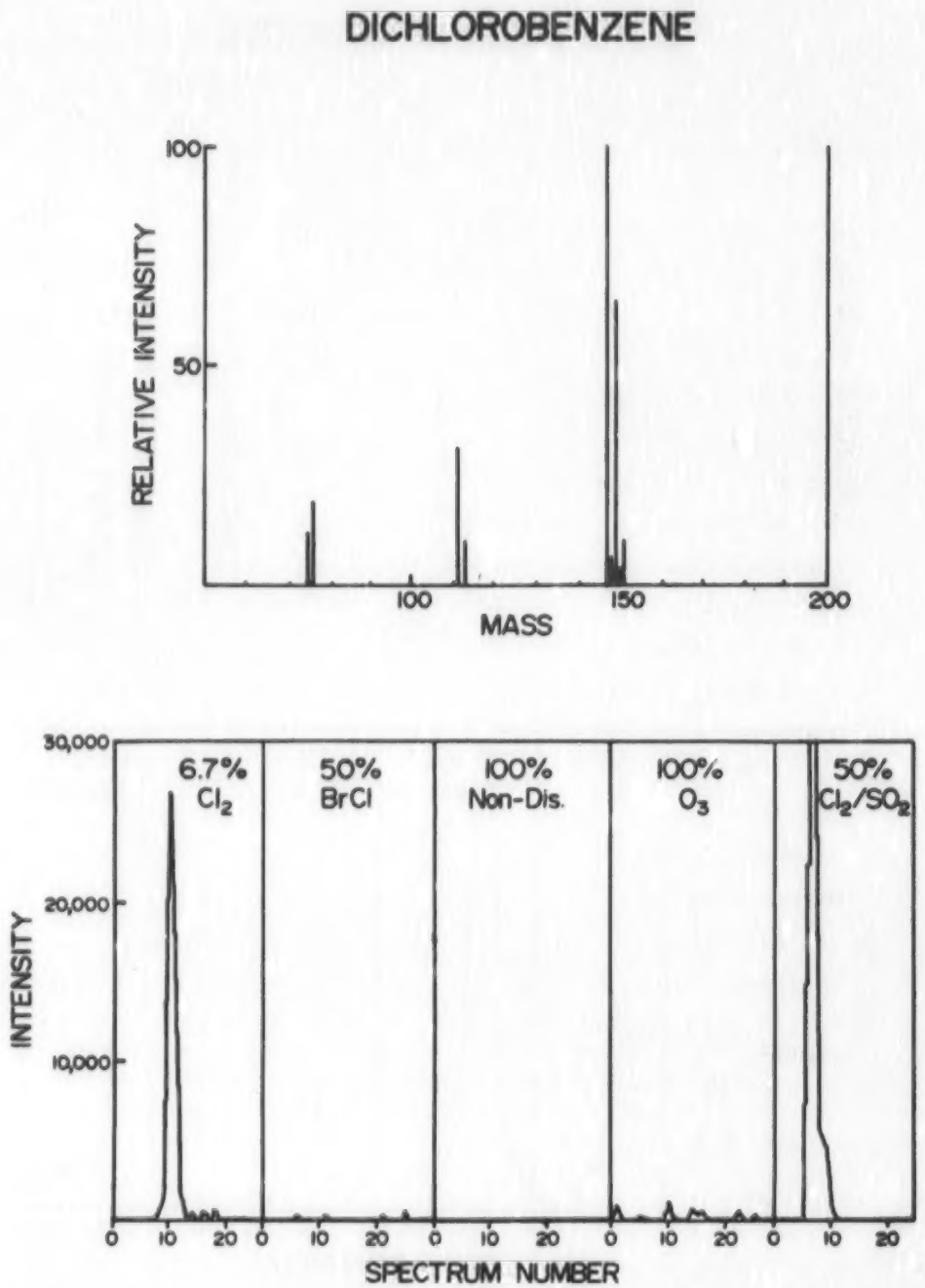

Fig. 2. Mass spectrum and reconstructed mass chromatograms (M^+ 146) of dichlorobenzene found in fish raised in chlorinated sewage effluent. The disinfectant and percent effluent concentration are given in the lower figure. The spectrum number is a guide to the gas chromatographic retention time.

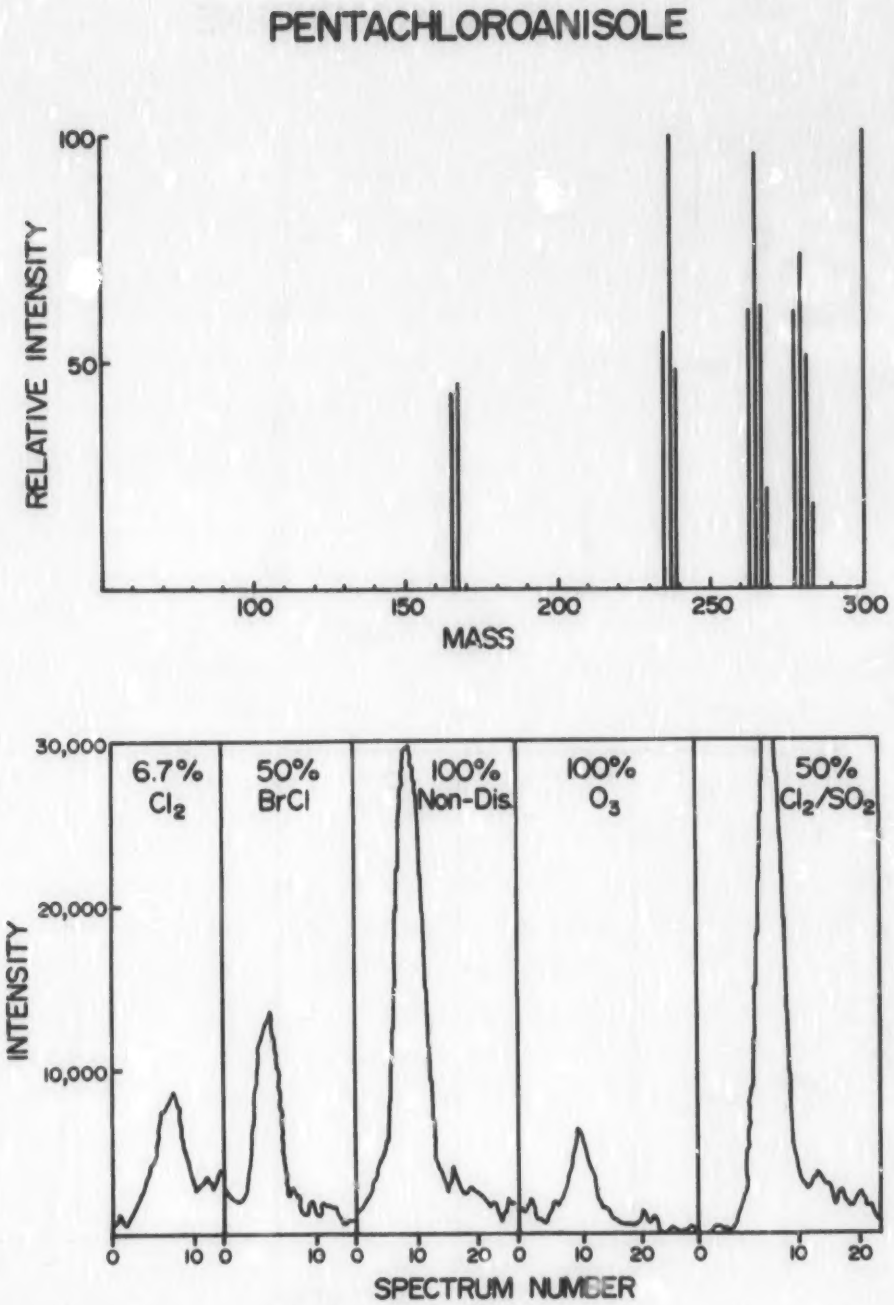

Fig. 3. Mass spectrum and reconstructed mass chromatogram (M^+ 278) of pentachloroanisole found in fish raised in sewage effluent. The disinfectant and percent effluent concentration are given in the lower figure. The spectrum number is a guide to the gas chromatographic retention time.

It is apparent that some compounds have a greater tendency to accumulate in the tissue than others. This phenomenon has been investigated by Macek et al. (1975) and Neely et al. (1974). They have shown that bioaccumulation is directly related to the partition coefficient (P) of the compound.

The use of the *n*-octanol/water partitioning system in correlating observed responses to compounds is not new. In 1899 Overton and Meyer correlated the solubility characteristics (*n*-octanol/water partition coefficient, P) of anesthetics to their observed biological response (log 1/C) (Overton 1899, Meyer 1899, Meyer and Hem[illegible] 1935, Meyer 1937):

$$\log 1/C = a \log P + b \quad . \tag{1}$$

In this equation C is the molar concentration of a given compound required for a particular response (narcosis, LC50, LD50). This original work dealt with isonarcosis in tadpoles, and the chemicals studied were alcohols, ketones, esters, and aromatic hydrocarbons. Ferguson (1939) placed the Overton-Meyer theory on a quantitative basis by defining the free energy change, ΔG, in terms of the chemical potential, μ.

In the 1960's Hansch and coworkers (1969) began applying these theories and were able to correlate many of the previously reported data by using the *n*-octanol/water system as the basic criterion. Electronic and steric factors have since been included to improve the correlation. Hansch introduced the need of a parabolic relationship in addition to a straight line relationship because as a compound became more lipophilic (higher partition coefficient, P) it tended to remain in the membranes and not be transferred to the active site and therefore caused a decrease in the observed activity. A parabola would be the best empirical representation for this type of observed biological response.

The onset of bioaccumulation is observed when the compounds start to remain in the cell membranes. It has been shown that the most likely place for the accumulation of lipophilic compounds is in the lipids of the animal, fish, or bird. At this point these compounds are transferred from water to birds throughout the environment by the food-chain mechanism.

Kapoor et al. (1973) studied food-chain mechanisms extensively and have shown how various chemicals are transported by this means. Bemelmans and ten Noever de Brauw (1974) observed this process in chickens that had been fed contaminated food. The contamination involved chloroanisoles of the same type as those isolated from the fish raised in the chlorine-treated effluent from Grandville, Michigan. Chlorinated anisoles have been reported (Engel et al. 1966, Curtis et al. 1972, Cserjesi and Johnson 1972) to impart a distinct musty taint to both eggs (yolks) and flesh of chickens. One source of the contamination was the wood shavings in the poultry houses, which were produced from lumber treated with technical grade pentachlorophenol (impurities constitute mainly isomeric tetrachlorophenols). The bacteria in the poultry litter were shown to be capable of methylating the chlorophenols on the surface of the wood shavings. It was also shown that some bacteria were capable of dechlorinating the higher chlorinated phenols. The problem persisted even when wood shavings were not used. This resulted in the discovery that the poultry feed was contaminated with trichloroanisole, which has a very low taste threshold (Table 6).

Table 6. Taste detection threshold of compounds in aqueous solution[a]

Compound	Concentration (µg/g)
Pentachloroanisole	4×10^{-3}
2,3,4,6-tetrachloroanisole	4×10^{-6}
2,4,6-trichloroanisole	3×10^{-8}
2,3,6-trichloroanisole	3×10^{-10}

[a]Curtis et al. 1972.

Bemelmans and ten Noever de Brauw (1974) were unable to ascertain the cause of the tainted feed. Investigation has determined that fish products have been used in various formulations of chicken feed and in light of the contaminated Grandville fish it is highly possible that contaminated fish could have been involved. Thus aqueous chlorination may create a problem as large as the initial one that required its use as a disinfectant.

Anisoles are more persistent as compared to the parent phenols. The methylation of phenol increases the partition coefficient from 1.48 to 2.04. Chlorination also increases the partition coefficient (0.7 units for aromatics and 0.4 units for aliphatics). Both of these increase the bioconcentration factor.

Neely et al. (1974) have shown with trout (*Salmo gairdneri* Richardson) that the bioconcentration factors of organic compounds when correlated to their *n*-octanol/water partition coefficients (P) resulted in a straight line (equation 2):

$$\log (\text{bioconc. factor}) = 0.542P + 0.124 \quad r = 0.948 \quad s = 0.342 \qquad (2)$$

In their study a series was chosen so that the partition coefficients were spread over a wide range. The bioconcentration factor was determined by the ratio of the rate of chemical uptake (k_1) in the test solution to the rate of chemical clearance (k_c) in fresh water. Figure 4 gives a graphical representation of their results. Table 7 illustrates the predictive application of equation 2. Mosquitofish (*Gambusia affinis*) were used for these bioassays, which demonstrate the importance of the use of partition coefficients as a predictive tool.

A standard approach to determining the bioconcentration factor should be defined so that data can be reported in a standard way and more important can be more easily compared to previously reported data. A reasonable standard expression would be the ratio of the initial uptake rate (k_1) in the test solution to the clearance rate (k_c) in fresh water. With this in mind three basic patterns have been observed during a 28-day exposure: (1) $k_1 > k_c$, continuous increase in residue concentration during exposure; (2) $k_1 \approx k_c$, initial residue concentration increase, until an unchanging level is observed; and (3) $k_1 < k_c$, initial residue concentration increase followed by a continuing decrease in concentration with exposure time. The initial rate constant will be dependent on the partition coefficient (P) and independent of k_c because the compound has not reached the site of action in large enough quantities to be of concern under the conditions of the bioassay. In order that k_c

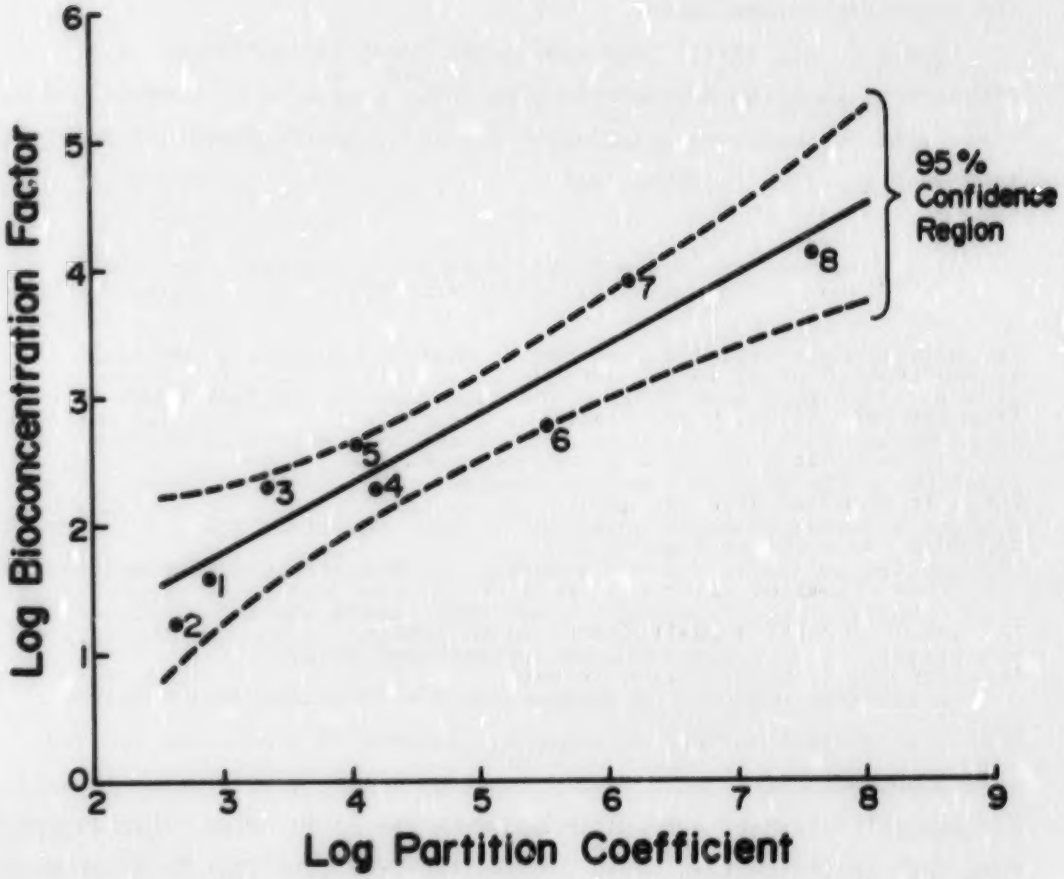

Fig. 4. Linear regression between logarithms of n-octanol-water partition coefficient and bioconcentration of chemicals in trout muscle (Neely et al. 1974). Compounds plotted are: (1) 1,1,2,2-tetrachloroethylene; (2) carbon tetrachloride; (3) *p*-dichlorobenzene; (4) diphenylether; (5) diphenyl; (6) 2-biphenylphenylether; (7) hexachlorobenzene; and (8) 2,2',4,4'-tetrachlorodiphenylether.

Table 7. The use of regression equation 2 for predicting the bioconcentration factor[a]

Chemical	Log partition coefficient	Log bioconcentration factor	
		Calculated	Experimental
Endrin	5.6	3.47 ± 0.989	3.17
Chlorpyrifos[b]	4.82	2.87 ± 0.963	2.67
3,5,6-trichloro pyridinol	1.35	0.88 ± 1.139	0.49

[a] Neely et al. 1974.

[b] 0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl)phosphorothioate.

be independent of k_1 in the second step, the fish have to be removed from the test solution and placed in fresh water. In this way one should be able to observe only the clearance mechanism. There may be several steps in each of these two general mechanisms, but the rate should be dependent only on the slowest step.

These examples illustrate some of the possible ramifications of the use of partition coefficients in an attempt to predict not only toxicity but bioaccumulation as well.

SUMMARY

A method is needed to rapidly screen the large number of organic compounds which if allowed to be discharged will contaminate our environment. The pharmaceutical industry has used water-solvent partition coefficients with great success in determining which compounds might be most biologically active. These coefficients can also be used as a predictive tool in the environmental world to study both toxicity and bioconcentration of organic contaminants. Unfortunately, the problems that arise with bioaccumulation of organic compounds which have been discharged into the environment are not immediately apparent. By the time a problem is identified it is usually too late and a major segment of the ecosystem has been contaminated. New products should be

manufactured with this in mind, and a complete data base of bioaccumulation data needs to be developed to set reasonable guidelines which industry can use.

The incorporation of chlorine into compounds during the disinfection of waste effluents with chlorine is an undesirable end result of effluent treatment in that compounds become more persistent and bioaccumulate to a greater extent.

EXPERIMENTAL

Compound library generation. A large number of compounds which were predicted to be found in the chlorinated effluent were purchased or prepared by one of the following two procedures:

Procedure A. Five hundred milligrams of the starting organic compound was dissolved in 20 ml $CHCl_3$. Chlorine gas was bubbled into the solution at room temperature for 30 min. The excess chlorine was then stripped off with $CHCl_3$

Procedure B. Five hundred milligrams of the starting organic compound was dissolved in 20 ml CCl_4 Chlorine gas was bubbled into the solution at room temperature for 30 min. The solution was then irradiated with UV light for 10 min at 254 nm. The excess chlorine was then stripped off with CCl_4.

GC retention time and mass spectral data were then obtained on each product. The mass spectra were loaded onto magnetic tape for library searches during sample investigations.

Water Analysis. The three water samples taken for examination were: (1) 8.0 gal of nondisinfected effluent, (2) 9.0 gal of chlorinated effluent, and (3) 4.5 gal of chlorinated effluent adjusted to pH 2.0. Samples were obtained by running the desired effluent through a glass column (2.5 x 20 cm) filled with a 50-ml bed volume of XAD-2 resin (Rohm and Haas). Each column was washed with 200 ml of 0.1 *M* NaOH, 200 ml 0.1 *M* HCl, and finally 200 ml of methanol. The acid and base washes were neutralized and extracted with three 50-ml portions of ether. The extracts were concentrated to 10 ml and the base extract methylated with diazomethane for GC and GC/MS analysis.

Fish Analysis. Whole fish (30 to 50 g) were blended with enough anhydrous sodium sulfate to make a dry powder and extracted with four 100-ml portions of hexane/ether (1:1). The extracts were concentrated, and the resulting oil residue was diluted with cyclohexane to 1 g/5 ml. The oil residue solution was placed on a 2.5-cm x 30-cm SX-2 (Bio-Rad Laboratory) gel permeation column and eluted with cyclohexane at 3.5 ml/min. The first 150 ml was vented, and the next two 60-ml fractions were collected. The collected fractions were combined and concentrated by using a Kuderna-Danish flask fitted with a three-ball Snyder column. Volumes were adjusted with hexane for GC and GC/MS analysis. Fish were obtained from the following bioassay tanks: (1) 6.7% Cl_2, (2) 50% Cl_2/SO_2, (3) 50% BrCl, (4) 100% O_3, and (5) 100% nondisinfected.

Instrumental Conditions. All gas chromatograms were run on a Varian Areograph 1700 equipped with a 6-ft x 1/8-inch glass column packed with 3% OV-101 on 80/100 mesh gas chrom Q. All GC effluent was monitored by electron capture-flame ionization detectors with a 1:50 splitter. Injection port and detector block temperatures were maintained at 230°C.

The mass spectrometer system was a Varian MAT CH-5 equipped with a Varian MAT mass spectrosystem 100 MS data system. All mass spectra were obtained at 70 eV with a 5-sec-per-mass decade scan.

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DISCUSSION

Alan H. Molof, Polytechnic Institute of New York. There are two parts to my question. First, in your testing protocol, did you use a clean tap water with the disinfectant you used for the activated sludge effluent? This would be valuable since it would relate to the effect of the disinfectants themselves, such as the effect of chlorine itself on fish. Second, have you or anyone else looked into the effects of disinfectants themselves in drinking water on man? Even though the conference title is "Environmental Impact of Water Chlorination," there does not seem to be any coverage of this subject. The importance of this area comes from the use of chlorine residuals in our tap water.

Kopperman. Well, again it comes to grant money. I am assuming such studies are being done in other places such as at the EPA Laboratory in Cincinnati.

Herman F. Kraybill, National Cancer Institute. Bioaccumulation and partition coefficients cause a problem in bioassay for carcinogenicity of organochlorine compounds. In setting an MTD (maximum tolerated dose) one proceeds for 6 to 7 months in the test and then the rodents being tested are killed due to the concentration increase in the adiposity. Even at 0.5 MTD, lethalities occur several months later and one is not able to pursue the bioassay. One has to start over at much lower doses where the bioaccumulation will not overload the metabolism.

Kopperman. Actually all of us are a little overweight. We have probably stored more than our share of some of the chlorinated compounds. All sorts of things might happen if we were to go on crash diets. These compounds have to go somewhere when you start getting rid of your fat tissue. They go right into your system.

Jerry J. Nelson, U.S. Energy Research and Development Administration. You have what appears to be classic linear first order kinetics. Have you made any attempt to build a model for the uptake and movement such as a Van Dyne type descriptive model?

Kopperman. No. I have not because as it turns out I am no longer associated with EPA. I am not presently funded to do this type research.

Nelson. In addition, the slide showing initial uptake rates which overshoot and decay may involve two processes such as sorption and intake with subsequent desorption. Or do you think this perhaps is decay in addition to clearance due to metabolic decomposition or some such mechanism?

Kopperman. Yes, those are possible mechanisms.

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